Cell Host & Microbe
Previews

Staphylococcus and the Healing Power of Pus

David G. Russell[1,*]
[1]Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA
*Correspondence: dgr8@cornell.edu
DOI 10.1016/j.chom.2008.02.008

Staphylococcus aureus infections induce formation of neutrophil-rich abscesses filled with debris from dead phagocytes. Corbin and colleagues report that this pus has antimicrobial powers through the activity of calprotectin. Calprotectin, a member of the S100 family of proinflammatory proteins, acts through chelation of manganese. As manganese is an essential cofactor for several enzymes in *S. aureus*, this impacts bacterial growth and the bacterium's ability to withstand oxidative stress.

Staphylococcus aureus is a ubiquitous bacterium that is generating increasingly bad press coverage due to its propensity to adopt a pathogenic lifestyle in hospital and community settings. It colonizes the skin readily and can lead to a wide range of pathological conditions from, skin lesions to osteomyelitis, endocarditis, and septicemia. Drug resistance among *Staphylococcus* isolates amplifies this escalating problem (de Lencastre et al., 2007). In the 1960s, penicillin-resistant strains started to emerge. These are known as methicillin-resistant *S. aureus* (MRSA) and were found to show broad-spectrum resistance to all β-lactam antibiotics. MRSA have now given rise to multidrug-resistant strains that are impervious to macrolides, tetracyclines, and gentamycin. The presence of these new generations of MRSA in healthcare facilities is a serious concern globally.

Understanding the transition from commensalism to the adoption of a pathogenic lifestyle requires an appreciation of both the opportunities that a commensal bacterium may seek to exploit, and the "failure" of human defense mechanisms to deny the bacterium these opportunities. The elegant paper by Corbin and colleagues, published in a recent issue of *Science*, highlights this transition by elucidating the crosstalk between bacterial physiology and the innate defense mechanisms of the host (Corbin et al., 2008).

One of the pathological consequences of an *S. aureus* infection is the development of skin abscesses characterized by the extensive recruitment of neutrophils. Neutrophils are the front-guard in response to bacterial invasion and are equipped with an arsenal of antimicrobial agents. They generate a robust superoxide response, contain a range of antimicrobial peptides, and can degranulate to release a toxic mix of hydrolases. A horde of angry neutrophils is usually damaging to both pathogen and host, and this extreme reaction is, of necessity, transient in nature. It is regulated by the developing acquired immune response, which combats the infection and limits tissue damage. Any pathogen capable of surviving this frontal assault will be met by a less frenetic, but more focused attack mediated by antibodies, lymphocytes, and macrophages.

Corbin and colleagues have made some observations key to our understanding of the mode of action of the neutrophil-derived, antibacterial protein calprotectin (Corbin et al., 2008). Their data stress the importance of this protein in restricting bacterial replication within the infected lesion. Calprotectin is a heterodimer known by several different names: S100A8/S100A9, MRP8/14, calgranulin A/B, and 27E10 antigen (Striz and Trebichavsky, 2004; Yui et al., 2003). It is an EF-hand, Ca^{2+}-binding protein that accounts for a staggering 40% of the total cytoplasmic protein concentration of neutrophils. It is known for its ability to inhibit the growth of a broad range of microbes, including both fungi and bacteria, and was thought to function through the chelation and sequestration of Zn^{2+}. The authors confirmed that calprotectin suppressed growth of *S. aureus* through the chelation of an essential component in the media; however, they went on to demonstrate that it was Mn^{2+}, rather than Zn^{2+}, that rescued the bacteria from growth limitation.

Manganese is a particularly intriguing target because, in addition to being an essential cofactor for catalases, superoxide dismutases, and peroxidases, Mn(II) is also capable of acting catalytically to scavenge superoxide and hydrogen peroxide (Horsburgh et al., 2002b). The authors confirmed the central role of Mn^{2+} in limiting bacterial growth through the demonstration that *S. aureus* mutants lacking in Mn^{2+} transport (*mntA* and *mntB*) showed increased sensitivity to calprotectin, while mutants that suffered from Mn toxicity because they were unable to regulate Mn^{2+} transport (*mntR*) were protected by calprotectin. Following phagocytosis of the cocci, neutrophils from wild-type and calprotectin-deficient mice were able to kill *S. aureus* equally well. However, cytoplasmic extracts from human neutrophils inhibited bacterial growth, and this activity was reversed by either immunodepletion of calprotectin or addition of excess Mn^{2+}. As neutrophil-rich lesions, such as the *S. aureus* abscesses, exhibit massive cell death of recruited phagocytes, it is not difficult to see how this protein could accumulate at the site of infection and act as a sink for available Mn^{2+}.

So, what are the consequences of calprotectin activity and Mn^{2+} depletion? This is where I believe it gets even more interesting and promises to be a fertile area for future research. In *S. aureus*, in addition to the need for Mn^{2+} as a nutrient, Mn^{2+} homeostasis is intimately interwoven with regulation of iron availability and the defense against oxidative stress (Horsburgh et al., 2002b), diagrammed in Figure 1. *MntR* encodes a Diphtheria toxin repressor (DtxR)-like protein that controls expression of the MntABC and MntH Mn^{2+} transport systems (Ando et al., 2003; Horsburgh et al., 2002a). Mutations

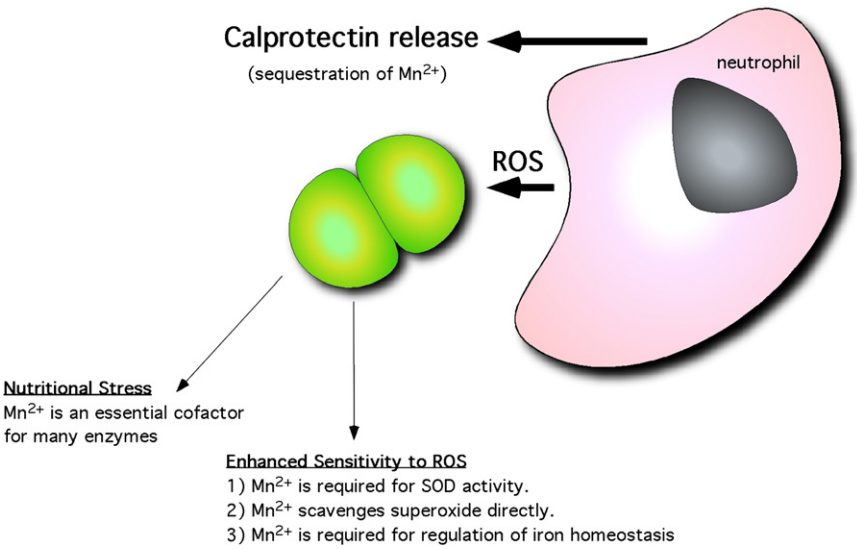

Figure 1. Neutrophils Are Recruited to the Site of Infection, Where Many of the Cells Die through Programmed Cell Death and Necrosis

Death of the neutrophils releases calprotectin, which is abundant in the cytosol of these phagocytes. Calprotectin acts to chelate manganese from the surrounding environment. The reduction of the concentration of available manganese diminishes the viability of *Staphylococcus aureus* through a variety of possible routes linked to nutrition and the capability of the bacterium to mount an effective response to the reactive oxygen species generated by the neutrophils.

in *mntABC* were reported to increase the sensitivity of the bacteria to reactive oxygen species (ROS), a phenotype that can be rescued by addition of Mn^{2+} (Horsburgh et al., 2002a). *PerR*, the peroxide resistance regulon repressor, is a Mn^{2+}-dependent, peroxidase-sensitive regulator that modulates *mntABC* expression, along with the expression of *katA* (catalase), *ftn* (ferritin), and *fur* (ferric uptake regulator) (Horsburgh et al., 2002b; Morrissey et al., 2004). This cascade suggests that chelation of Mn^{2+} by calprotectin will impact *S. aureus* survival through denying the bacterium an essential cofactor, while rendering it potentially more susceptible to the reactive oxygen intermediates released by the neutrophils. This susceptibility may be mediated through multiple routes, such as inhibition of Mn^{2+}-dependent superoxide dismutase activity (Karavolos et al., 2003), and absence of Mn(II)-mediated scavenging of superoxide and hydrogen peroxide (Horsburgh et al., 2002a). The reduced availability of Mn^{2+} may also diminish PerR activity, upregulating expression of Fur, which should lead to decreased iron transport and increased expression of catalase and ferritin (Horsburgh et al., 2001). In theory, this should lead to a decreased susceptibility to ROS; however, this is predominantly the route of avoiding damage mediated by H_20_2, which won't be generated in the absence of superoxide dismutase. Additional studies are clearly needed to determine by which route(s) the depletion of Mn^{2+} mediated by calprotectin may impact the bacterium's resistance to oxidative stress generated by the neutrophil's membrane-associated NADPH oxidase complex.

Finally, Paclet and colleagues recently reported that the NADPH oxidase complex isolated from B cells showed a lower constitutive turnover rate of O_2^- production than the oxidase complex isolated from neutrophils (Paclet et al., 2007). Upon analysis they determined that the conformation of the NADPH oxidase-associated membrane cytochrome b_{558} from neutrophils was in an "open" state that enhanced the activity of the complex. The addition of the calprotectin dimer to purified NADPH oxidase from B cells altered the conformation of cytochrome b_{558} and enhanced the output of O_2^-. This indicates that calprotectin may enhance killing both by rendering the pathogen more susceptible and by enhancing the output of ROS from the neutrophil.

The work of Corbin and colleagues opens the way to dissecting the mechanisms behind calprotectin's activity and brings a new appreciation of the healing power of pus. Moreover, the sequestration of manganese as a means of limiting bacterial growth could have broader applications in controlling bacteria on both biological and nonbiological surfaces. This paper opens up many new avenues of speculation and experimentation!

REFERENCES

Ando, M., Manabe, Y.C., Converse, P.J., Miyazaki, E., Harrison, R., Murphy, J.R., and Bishai, W.R. (2003). Infect. Immun. *71*, 2584–2590.

Corbin, B.D., Seeley, E.H., Raab, A., Feldmann, J., Miller, M.R., Torres, V.J., Anderson, K.L., Dattilo, B.M., Dunman, P.M., Gerads, R., et al. (2008). Science *319*, 962–965.

de Lencastre, H., Oliveira, D., and Tomasz, A. (2007). Curr. Opin. Microbiol. *10*, 428–435.

Horsburgh, M.J., Ingham, E., and Foster, S.J. (2001). J. Bacteriol. *183*, 468–475.

Horsburgh, M.J., Wharton, S.J., Cox, A.G., Ingham, E., Peacock, S., and Foster, S.J. (2002a). Mol. Microbiol. *44*, 1269–1286.

Horsburgh, M.J., Wharton, S.J., Karavolos, M., and Foster, S.J. (2002b). Trends Microbiol. *10*, 496–501.

Karavolos, M.H., Horsburgh, M.J., Ingham, E., and Foster, S.J. (2003). Microbiology *149*, 2749–2758.

Morrissey, J.A., Cockayne, A., Brummell, K., and Williams, P. (2004). Infect. Immun. *72*, 972–979.

Paclet, M.H., Berthier, S., Kuhn, L., Garin, J., and Morel, F. (2007). FASEB J. *21*, 1244–1255.

Striz, I., and Trebichavsky, I. (2004). Physiol. Res. *53*, 245–253.

Yui, S., Nakatani, Y., and Mikami, M. (2003). Biol. Pharm. Bull. *26*, 753–760.